

Spectral reflectance of duckweed (*Lemna gibba* L.) fronds exposed to ethylene glycol

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Abstract

When duckweed (*Lemna gibba* L.) fronds are exposed to ethylene glycol (EG), internal anatomy is altered, allowing an increase in water uptake that causes a darkening of frond appearance. Spectroradiometry was used to quantify changes in frond reflectance that occurred throughout the 400-850 nm spectrum under various EG concentrations and exposure times. The threshold concentration of EG at which a reflectance change could be detected was between 35 and 40 mM ($P \leq 0.01$), approximately the same as by visual observation. EG-induced changes in frond reflectance were maximum at concentrations of 50 mM or greater. Reflectance changes were detectable within 24 h of exposure to 100 mM EG, 2-3 days prior to changes in frond appearance. The spectroradiometry of duckweed may serve as a rapid and sensitive technique for detection of ecosystem exposure to EG and perhaps other stress agents.

Keywords: Duckweed, Ethylene glycol, *Lemna gibba*, Spectrum, Reflectance

1. Introduction

Ethylene glycol (EG) is a very common anthropogenic chemical. In 1995, 2.37 billion kg of EG were produced in the US alone, ranking it in the top fifty chemicals produced (Kirschner, 1996). It is used extensively by various industries worldwide, most notably as an automobile engine coolant and aircraft deicing agent (Sills and Blakeslee, 1992). As a result, large quantities of EG enter the environment (Hartwell et al., 1995). The various uses of EG virtually guarantee that it is a common contaminant of surface waters. However, it is usually considered to be a benign pollutant because of its relatively low toxicity to animals and microorganisms (Bridie et al., 1979; Bringman and Kuhn, 1980; Pillard, 1995), and because it can be metabolized by various aerobic (Child and Willets, 1978; Willets, 1981) and anaerobic bacteria (Strab and Schink, 1986; Tanaka, 1990).

1 Consequently, few published studies have considered the effects of EG on higher plants,
2 including aquatic angiosperms. This is somewhat surprising since aquatic organisms are
3 among the first to be exposed to the EG carried in storm water runoff.

4 The duckweeds (Lemnaceae) provide ideal models for studying the effects of EG on
5 aquatic angiosperms. They are the smallest and simplest flowering plants (Hillman, 1961)
6 and have a rapid growth rate in defined media under aseptic conditions. Reproduction is
7 almost invariably vegetative and thus large numbers of genetically homogeneous fronds
8 can be cultivated for study. Duckweed occurs worldwide (Hillman, 1961; Clark, 1979),
9 including every state in the U.S. (Muenscher, 1944). It is not only a staple food for ducks
10 (Clark, 1979) but also for other organisms including fish, crayfish and nutria (Chambers et
11 al., 1991; Wisley et al., 1991).

12 Previous study has demonstrated that EG induces changes in cell membrane
13 permeability and wall structure in axenically grown fronds of L. gibba (Barber et al., 1999).
14 Fronds exposed to EG take up increased amounts of water and water soluble
15 compounds. One effect of the increased water uptake is a change in frond optical
16 properties such that they assume a dark green, translucent appearance similar to that of
17 thawed spinach leaves. While such visual changes associated with exposure to EG are
18 obvious, they are not easily quantified. Although L. gibba and other duckweeds have been
19 used in many toxicological studies, it appears that responses of frond spectral reflectance
20 to physiological damage have not been evaluated previously. The results reported below
21 describe a method which quantifies EG-induced damage in terms of frond spectral
22 reflectance.

23 Leaf reflectance methods which have been used to indicate stress in terrestrial plants
24 (for review see Carter and Knapp, 2001) immediately suggested themselves as adaptable
25 for the measurement of EG-induced damage in L. gibba. Herein, we tested the hypothesis

1 that frond spectral reflectance in the 400-850 nm range would provide a means to quantify
2 EG-induced stress in *L. gibba*.

3

4 **2. Materials and methods**

5 Stock cultures of *L. gibba* (Kandeler's strain G3) were maintained axenically in 125 ml
6 Erlenmeyer flasks, each containing 50 ml of the growth medium described by Cleland and
7 Tanaka (1979). Sucrose and tryptone were added to reveal microbial contamination. The
8 stocks were grown under continuous illumination (2.0 mW cm^{-2} , 300-1100 nm at plant
9 level) at $27.8 \pm 1^\circ\text{C}$. Every 5 or 7 days new stocks were established by inoculating 10-20
10 fronds into fresh medium. Stock cultures no more than 7 days old (approximately
11 equivalent to a single layer of fronds) were used to initiate experimental cultures.

12 In preparing cultures for exposure to a range in EG concentration, EG (Fisher Scientific,
13 Pittsburgh, PA, USA) was sterilized by autoclaving at 138 kPa and 121°C for 20 min. After
14 cooling, the EG was added aseptically to 50 ml of sterile medium in each of several 125
15 ml Erlenmeyer flasks to give nominal EG concentrations of 10, 25, 30, 35, 40, 45, 50 and
16 100 mM. Approximately 15 parental fronds from a stock culture were inoculated into each
17 experimental flask. Fronds were also inoculated into control treatment flasks that
18 contained 0 mM EG. During incubation and culture growth, the parental fronds produced
19 daughter fronds by vegetative reproduction. Thus, fronds sampled after exposure to
20 treatment consisted of the original parental fronds mixed with daughter fronds. After 7
21 days of incubation, the parental and daughter fronds from each flask were removed and
22 placed in 3.5 cm Petri dishes to enable reflectance measurements as described below.

23 To determine the influence of EG exposure time on frond reflectance, approximately 15
24 parental fronds from a stock culture were inoculated into each of several flasks containing
25 50 ml of growth medium with either 0 or 100 mM EG. Reflectances of six replicate
26 samples were measured on days (24 h periods) 0, 1, 2, 4, 6 and 8 of exposure. On days

0, 1, 2 and 4, individual flasks did not contain a sufficient number of parental plus daughter fronds for reflectance measurements. Thus, fronds from several flasks were combined to form six measurable samples per treatment.

Radiances reflected throughout the 400 to 850 nm spectrum from sample fronds and a white reference (Spectralon SE-590, Labsphere, N. Sutton, MA, USA) were measured as described earlier (Carter et al., 1995) using a LI1800UW spectroradiometer with LI1800-06 microscope body and accessories (LI-COR, Lincoln, NE, USA). Fronds were placed in Petri dishes such that no gaps appeared between fronds and their upper surfaces faced the lens of the spectroradiometer. Previously, the dishes had been sprayed with flat black paint to minimize background reflectance at 1-5% throughout the 400-850 nm range.

Samples were irradiated using a 600 W tungsten lamp and a constant irradiance angle of 65° from the normal to minimize the measurement of direct reflectance, or glare, from the fronds. View angle of the spectroradiometer was normal to the surface of each Petri dish. An average spectral reflectance was measured for all fronds located within a circular field-of-view that was approximately 2 cm in diameter. No attempt was made to quantify the relative contributions of parental vs. daughter fronds to average reflectance in either experiment. An electric fan was used to dissipate heat from the lamp and reduce incidental heating of samples. True spectral bandwidth determined by the 0.5 mm slitwidth of the monochromator was 4 nm. Data were output at 1 nm intervals throughout the 400-850 nm spectrum. Spectral reflectances in units of percent were computed by multiplying frond reflected radiances by 100 and dividing by the reference reflected radiance at each 1 nm wavelength interval.

At each 1 nm wavelength interval, reflectance for each treatment was compared to control reflectance using a general linear models procedure (GLM procedure, SAS v. 6.0, SAS Institute, Cary, NC, USA). Mean reflectance differences as defined in Carter and Knapp (2001) were obtained by subtracting mean control reflectance from the mean

reflectance of each other treatment. When $P \leq 0.01$ for the F statistic, the reflectance difference in that waveband was regarded as significant.

3. Results

Regardless of EG concentration, the shapes of frond reflectance curves were characterized by peaks in the green spectrum near 550 nm and in the near-infrared beyond 720 nm (Fig. 1). As EG concentration increased, reflectance tended to decrease throughout the 450-850 nm spectrum. However, reflectance differences between treated and control fronds did not become significant ($P \leq 0.01$) until EG concentration reached 40 mM (Fig. 2). The wavebands in which significant differences occurred were 485-850 nm at 40 mM, 445-850 nm at 45 mM, 432-850 nm at 50 mM, and 412-850 nm at 100 mM EG.

Increasing the duration of exposure to 100 mM EG produced changes in frond reflectance that were similar to those produced by increasing EG concentrations (Fig. 3). After one day of exposure, significant reflectance differences were evident only in the narrow 723-739 nm waveband (Fig. 4). Following a second day of exposure to EG, reflectance differences occurred throughout the near-infrared at 711-850 nm. After 4, 6 and 8 days of exposure, differences were significant throughout most of the spectrum at 429-850 nm, 432-850 nm and 428-850 nm, respectively.

4. Discussion

Spectral reflectances of Lemna fronds (Figs. 1, 3) were characteristic of green leaves in general (Gates et al., 1965; Knapp and Carter, 1998). Low reflectances in the blue and red spectra result from strong absorption by chlorophyll, whereas peak reflectance near 550 nm results from a reduced chlorophyll absorptivity in the green spectrum. Leaves do not contain substances that absorb strongly in the near-infrared, resulting in high

1 reflectances beyond approximately 720 nm (Gates et al., 1965). At all wavelengths in the
2 incident solar spectrum (ca. 400-2,500 nm), light is partially reflected from interfaces
3 between wet cell walls and intercellular air as a result of discontinuities in refractive index
4 (Knippling, 1970; Woolley, 1971; Sinclair et al., 1973; Tucker and Garratt, 1977). These
5 multiple reflections result in a high light intensity within the leaf (Vogelmann, 1993) and
6 reflectances of 50% or more at weakly absorbed wavelengths such as in the near-
7 infrared.

8 Fronds of L. gibba are normally constructed of aerenchymatous tissues composed of
9 uninterrupted, unicellular layers of chlorenchyma cells that surround large, air-filled
10 spaces (Barber et al., 1999). Fronds exposed to EG are constructed similarly except that
11 the chlorenchyma layers surrounding the air spaces are interrupted by gaps with
12 diameters as large as ca. 10 μm . These gaps allow water to infiltrate the fronds (Barber et
13 al., 1999), greatly reducing the numbers of refractive index discontinuities. Thus, multiple
14 reflections within the leaf are reduced and reflectance is decreased throughout the
15 incident spectrum. This effect is apparent to the naked eye in the dark green, translucent
16 appearance of the EG-treated fronds. The similar molecular structures of EG and
17 ethylene, a known phytohormone, lead to speculation that EG may exhibit some hormonal
18 behavior in initiating the anatomical changes which result in excessive water uptake.
19 However, only those fronds produced under exposure to EG are structurally altered and
20 assume the dark green appearance. The parental fronds, and immature daughter fronds
21 contained in parental reproductive pockets, are unaffected when exposed to EG (Barber
22 et al., 1999).

23 It is a reasonable assumption that physiological stress in aquatic angiosperms, including
24 duckweed, is induced frequently in the environment by exposure to EG. Thus, it may be of
25 considerable importance to derive remote sensing methods for the early detection of
26 damage induced by EG. In terrestrial plants, subtle changes in leaf reflectance can

1 indicate damage that results from a variety of causes prior to visible changes in leaf color
2 (Cibula and Carter, 1992; Carter et al., 1996). However, these methods rely on stress-
3 induced reductions in leaf chlorophyll concentration rather than alterations in leaf
4 anatomy. Thomas (1998) demonstrated that pigment quantity and quality remained
5 unchanged in *L. gibba* following exposure to EG. Thus, changes in pigmentation did not
6 influence results in the present case. Instead, it appears that EG-induced changes in frond
7 reflectance can be explained entirely by water infiltration.

8 With respect to EG concentration, a threshold of 35–40 mM was required to produce
9 significant changes in either frond spectral reflectance (Fig. 2) or appearance. Thus,
10 spectroradiometry provided no advantage compared with visual observation in detecting
11 EG-induced damage. However, with respect to exposure time, spectroradiometry detected
12 damage after only 24 h of exposure to EG (Fig. 4) compared with the 3–4 days required
13 for a change in frond appearance. At this time, significant changes in reflectance were
14 limited to a narrow waveband in which human visual sensitivity is quite low (723–739 nm,
15 Fig. 4). It should be emphasized that on Day 0, reflectance was measured for populations
16 composed entirely of parental fronds. On successive sampling days, the proportion of
17 daughter fronds increased. By the end of Day 1, the proportion of daughter fronds that
18 were initiated in the presence of EG would have been extremely small, yielding a
19 correspondingly small change in reflectance. With further increases in exposure time, the
20 broadening of the spectrum in which reflectance changed significantly resulted from the
21 gradually increasing proportion of daughter fronds in the total population.

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Figure captions

Fig. 1. Mean reflectances representative of Lemna gibba populations exposed to different concentrations of ethylene glycol for 7 days. From top to bottom, the curves represent ethylene glycol treatments at concentrations of 0, 40, and 100 mM. Each curve represents an average of 7 to 11 samples.

Fig. 2. Differences in mean reflectance ($N=7-11$) between control (0 mM ethylene glycol) fronds of Lemna gibba and fronds exposed to different concentrations (10, 25, 30, 35, 40, 45, 50 and 100 mM) of ethylene glycol for 7 days. Reflectance differences were computed by subtracting mean reflectance for the control treatment from mean reflectances of each other treatment. Darkened areas indicate wavebands in which reflectance differences were statistically different ($P \leq 0.01$).

Fig. 3. Mean reflectances representative of Lemna gibba populations after 0, 4, or 8 days of growth in a 100 mM ethylene glycol medium. Each curve represents an average of 6 samples.

Fig. 4. Differences in mean reflectance ($N=6$) between control (0 mM ethylene glycol) fronds of Lemna gibba and fronds grown for 1, 2, 4, 6, or 8 days in a 100 mM ethylene glycol medium. Reflectance differences were computed by subtracting mean reflectance for the control treatment from mean reflectances of each other treatment. Darkened areas indicate wavebands in which reflectance differences were statistically different ($P \leq 0.01$).

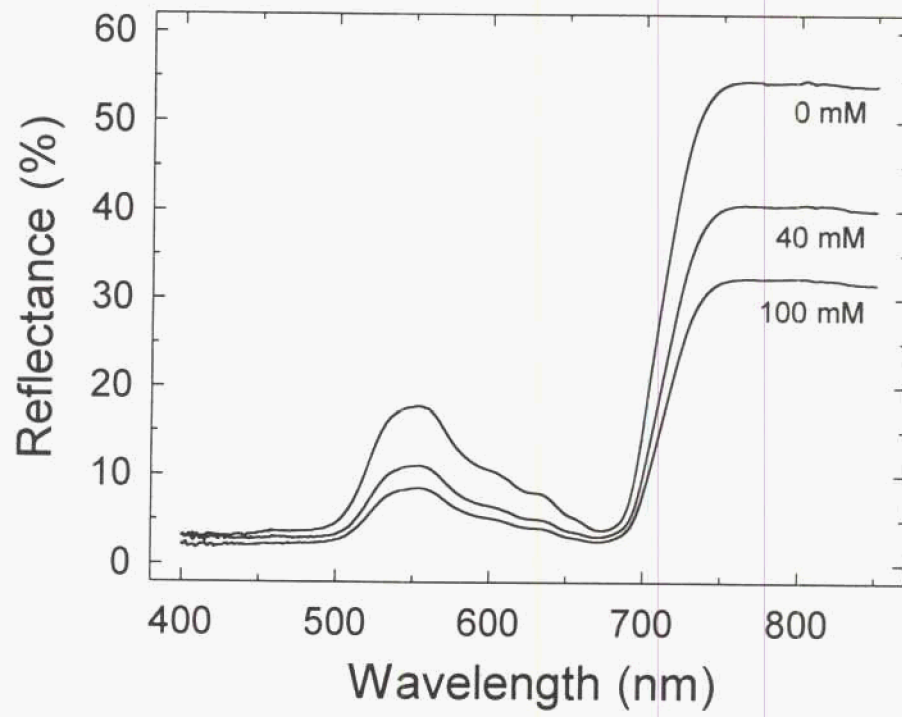


Fig. 1
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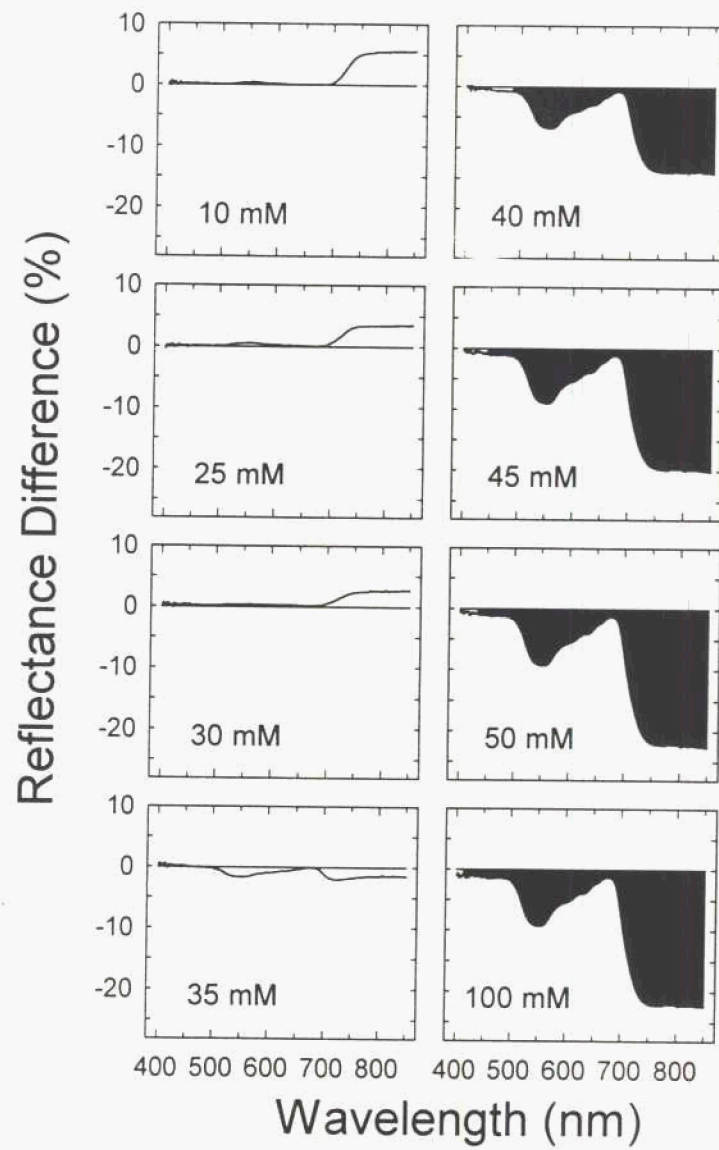


Fig. 2
Dong et al.

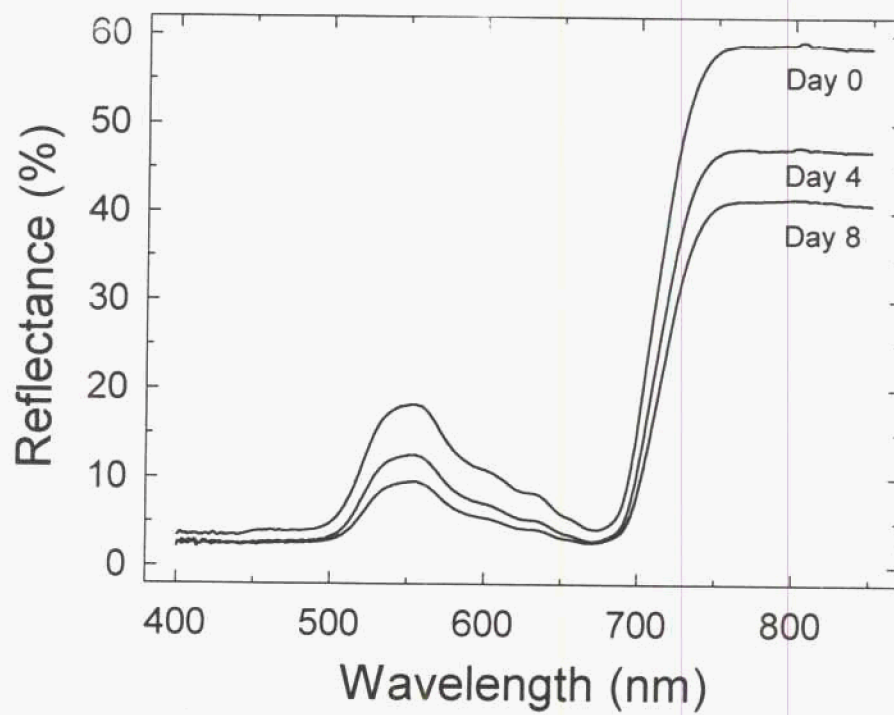


Fig. 3
Dong et al.

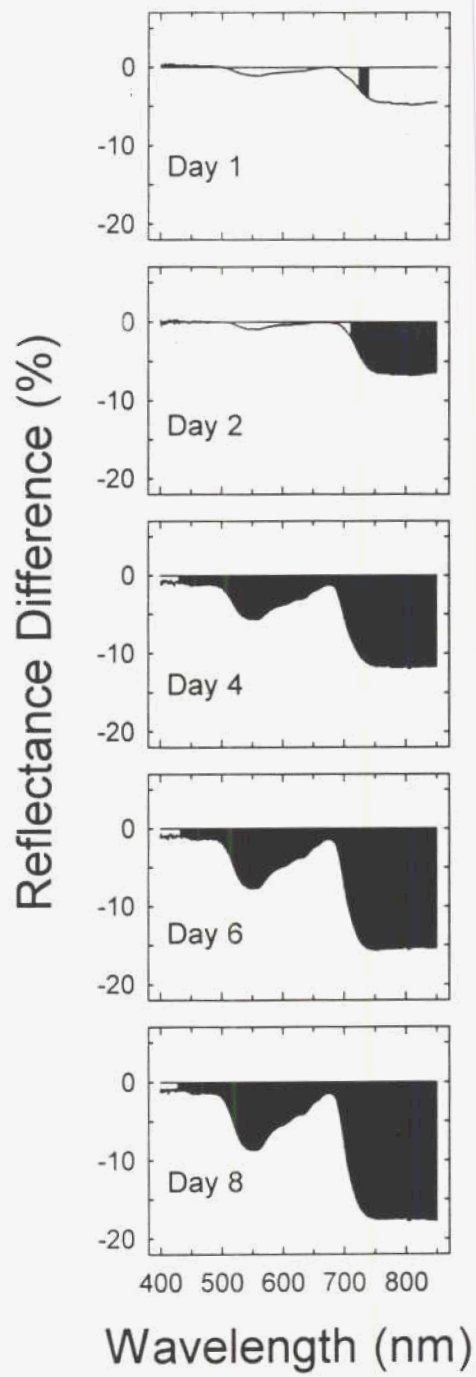


Fig. 4
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